Declaration under 37 C.F.R. § 1.132

I, Dr. Rainer Hintsche, declare and say:

1. I am a named inventor of the subject matter claimed in United States application serial no. 09/142,660 ("the Application").

2. I have received a Ph.D. in analytical organic chemistry and have worked in the field of biochemistry and biosensorics for 20 years. My curriculum vitae is attached as Appendix A.

3. I understand that, in one aspect, the claimed invention is directed to a method of detecting a molecule or molecule complex in a diluent, sovent or gel, including the steps of: (a) contacting the molecule or molecule complex with an ultra-microelectrode array that contains at least two electrode structures, wherein the spacing bewteen the electrode structures is less than 3 μm; (b) producing an alternating electric field between the electrode structures; and (c) measuring changes in current or potential between the electrode structures, whereby the changes in current or potential are caused by the molecule or the molecule complex ("the claimed invention").

4. I have reviewed the Office Actions dated December 8, 1999, and August 1, 2000, and I understand that the Examiner questions whether the specification adequately teaches a skilled worker how to make and use the full scope of the claimed invention.

5. I further understand that the basis for the Examiner's position is that the Application allegedly does not teach a

skilled worker how to accurately detect a molecule or molecule complex using an ultra-microelectrode array, except for β-galactosidase-streptavidin, which is illustrated by the "Illustrative embodiment," beginning at page 13, line 4 of the Application.

6. In my professional opinion, however, the guidance set forth in the Application—coupled with the techniques available at the filing date of the Application—allows a skilled worker in the field of biosensors or biochips through routine experimentation, to determine the conditions needed to accurately detect a given molecule (*e.g.*, nucleic acid or polypeptide) or molecule complex, using an ultra-microelectrode array. It requires only an adaption of basic procedures of common and commercialized microarrays or biochips or biosensors.

7. As objective evidence to support my statement in Paragraph 6, please find Examples 1-4 ("the Examples") appended hereto (Appendix B). The Examples show that I have been able to detect the hybridization of nucleic acids and polypeptides (other than those explicitly described in the Application), using the guidance set forth in the Application, in conjunction with the techniques available to a skilled worker in the field at the filing date of the Application.

8. I personally supervised different other workers of my group who performed the examples. These nucleic acids and polypeptides set forth in the Examples are representative of any given nucleic acid or polypeptide, as mentioned in Paragraph 6.

9. With reference to Appendix B, the following sub-paragraphs illustrate how a skilled worker can detect any number

of nucleic acids and polypeptides, based on the guidance provided in the specification:

(a) Example 1 relates to the detection of tumor marker CK-20 DNA, using impedance measurements.

(i) The chip was structured according to Figure 1 c and page 13, lines 5-6 of the Application. The chip electrodes were coated with a DNA probe, using conventional techniques [M.Hegner et al. FEBS Lett.336(1993)452-456; A.B.Steel et al. Biohpysical Journal 79(2000)975-981]. After recording an impedance spectrum, the coated electrodes were contacted with a sample of CK-20 DNA and subjected to conditions conducive to hybridization, followed by a wash.

(ii) The hybdridization conditions were configured by referencing [Synthetic oligo nucleotide probes - Chapter 11 Conditions for hybridization of oligo nucleotide probes - Sub-Chapter 11.45 In J. Sambrook, E. F. Fritsch, and T.Maniatis in *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Second Edition, New York, 1989]. Thereafter, impedance measurements were performed, using standard equipment. The measurements indicated an increased impedance when CK-20 DNA hybdridized to the probe that was coated on the electrode.

(b) Example 2 relates to the detection of FLAG antibody, using impedance measurement.

(i) The chip was structured according to Figure 1 of the Application. The surfaces of the electrodes were covered with a concentration of mercaptoundecanoic acid, as determined by [J.Spinke et al. J.Chem.Phys. 99(1993)7012-7019] which, in turn, resulted in a self assembled monolayer (SAM) on the electrode. The degree of electrode surface coverage was adjusted, using conventional electrochemical techniques [J.Wang et al. Anal.Chem. 65(1993)1893-1896]. Then, the carboxyl groups of the SAM were transformed into reactive esters, under methods well known to a skilled worker [H.G.Bäumert and H.Fasold "Crosslinking technniques" in Methods in Enzymology 172(1989)p 584 - 609, Academic Press,Inc.; V.M.Mirsky et al., Biosensors & Bioelectrocis 12(1997) 977-989], followed by the immediate addition of the peptide probe and incubation. After deactivating the remaining reactive esters using conventional means [H.G.Bäumert and H.Fasold "Crosslinking technniques" in Methods in Enzymology 172(1989)p 584 - 609, Academic Press,Inc.; V.M.Mirsky et al., Biosensors & Bioelectrocis 12(1997) 977-989], the impedance spectrum was measured.

(ii) For the formation of the affinity bound complex, a standard concentration of anti-FLAG antibody (Sigma F 3040, monoclonal mice IgG, Clone M1) was added to the immobilized probe-coated array and subject to a standard incubation protocol. [Sigma Aldrich, Inc.; Sigma product information: product code F 3040; US Patent 4,703,004]. Thereafter, the array surface was washed six times with a conventionally used buffer. The impedance measurements were performed as described for Example 1 and the measurement indicated the formation of an affinity binding complex on the array.

(c) Examples 3 and 4 are similar to Examples 1 and 2, respectively, except that, in simple terms, Examples 3 and 4 employ simultaneous electrode processes in addition to impedance measurement. In both Examples, the impedance measurements were recorded in the presence of redox species—potassium-hexacyanoferrat-(II) and (III)—using a common redox recycling technique as described by K.Aoki et al. in J. Electroanal. Chem. 256(1988)269-282 and taught by page 7, lines 5 through 18 of the Application.

10. All statements made herein of my knowledge are true and all statements made on information and belief are believed to be true; and further, these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or improsonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any document or any registration resulting therefrom.

Date:Itzehoe, Aug.9, 2001

Rainer Hintsche

Appendix A

Curriculum vitae

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Rainer Hintsche was born in Delitzsch, Germany, in 1942. He received his diploma in organic and analytical chemistry in the field of terpenes from the University of Leipzig in 1966 and his Ph.D. for a work of DNA-synthesis inhibiting nucleosides also from the University of Leipzig in 1970. From 1966 to 1969 he worked in Berlin towards his Ph. D. thesis at the Institute of Biochemistry in cancer research. From 1970 to 1983 he was a group leader in analytical chemistry at the Central Institute of Molecular Biology in Berlin. In 1986 he received a Habilitation degree (Dr.sc.) for the work of structure activity relationships of protein binding molecules. From 1983 to 1992 he was founder and leader of the chip-biosensor group at the Central Institute of Molecular Biology in Berlin.

In 1992 he joined together with his complete group the Fraunhofer-Institut für Siliziumtechnologie in Berlin, where he took over the position of the head of the department biotechnical microsystems. From 1995 this institute is located in Itzehoe (near Hamburg).

He is still engaged in electrical biochip technology for DNA- and protein chip arrays and chemical sensors in silicon technology using sensor sizes and and sensing interfaces in molecular dimensions. His furter interest is focussed on complete micro analysis systems including novel electrochemical detection procedures and related special integrated circuits.

He published 130 original papers and is inventor in 55 patents.

Awards: Award of the Eurosensors VI, San Sebastian, 1992;
Award of the 5th Int.Meeting on Chemical Sensors, Rome, 1994
Phillip Morris Prize, Germany 1996,

APPENDIX B

Example 1: **detection of tumor marker CK-20 DNA by impedance measurements:**

For the detection of tumor marker CK-20 DNA, a chip with an interdigitated gold electrode array (structured according to Figure 1c), an electrode width of 1µm and an electrode spacing of 0.7 µm was used for the probe immobilization. The electrodes surface was cleaned with concentrated sulfuric acid for 1 minute, washed with distillated water and then coated with 1µl of 1mM 5'-(SH-hexyl)-CGA TCT GTT TTA TGT AGG TCA-3' (probe DNA) solution in distillated water by means of self-assembling for 1 hour at room temperature. After washing with distillated water the array was immersed into phosphate buffer (pH=7.4). Then, an impedance spectrum was recorded.

The coated array was contacted with the DNA sample solution in a flow cell, under conventional hybridization conditions. Accordingly, a sample solution containing about 1µmol of the CK-20 DNA, which contained the target sequence 5'-Biotin-TGA CCT AAC CCT ACA TAA AAC AG-3' in 300mM NaCl, 30mM Na-Citrat-buffer (pH=7.0) was used. The surface was treated with 3µl of the solution for 30 minutes at 50°C - 60°C. After washing with 3mM Na-citrat-buffer with 30mM NaCl, pH=7.0 solution, the impedance of the array was measured again.

The impedance measurements were performed using a frequency response detector (model 1025) and a potentiostat/galvanostat (model 283 - EG & G Instruments Inc.), connected to a computer. The system was potentiostated at 200 mV vs. open circuit

potential. The amplitude of the AC input signal was 10 mV. Impedance spectra were recorded in the frequency range of 1 mHz - 1 MHz. The Nyquist plot indicated an increased impedance, indicating the formation of the double-stranded DNA on the array.

Example 2: **detection of FLAG antibody by impedance measurements:**

For the detection of "FLAG" antibody, which is a commercially available antibody against the below described synthetic peptide sequence; see Sigma Aldrich, Inc.; Sigma product information]an interdigitated gold electrode array, structured according to Figure 1, and having an electrode width of 2 µm and an electrode spacing of 0.8 µm was used for the probe immobilization. The array electrode surface was cleaned with concentrated sulfuric acid for 1 minute and washed with distillated water. The surface then was covered with 4 µl of a solution of 5 mM 11-mercaptoundecanoic acid in distilled H_2O, to allow for self assembling, which resulted in a self assembled monolayer (SAM).

After 30 minutes, the array was washed with distilled H_2O and dried. The amount of surface coverage was adjusted to be 80-90 %, as determined by the current response of redox recycling of 20mM p-aminophenol in phosphate buffer (pH 7.4). Then, the carboxyl groups of the SAM were transformed into reactive esters with 3 µl of a solution of 1 M benzotriazol-1-yloxy-tripyrrolidino-phosphonium-hexafluorophosphate (PyBOP) and 1 % (v/v) diisopropyl-ethyl-amine (DIEA) in dry dimethylformamide (DMF) for 15 min. After washing with 200 µl phosphate-buffered saline (PBS) (pH 7.4), 3 µl of a solution containing the peptide probe for the FLAG -sequence (N-Asp-Tyr-

Lys-Asp-Asp-Asp-Asp-Lys-C) at a concentration of 0.5 mg/l was quickly added to the activated SAM and incubated for 20 minutes. After washing with PBS, the remaining reactive esters were deactivated with 4 µl of a solution containing 3 % (w/v) glycine in PBS and 1 % (v/v) DIEA for 20 mininutes. After washing with distillated water, the array was immersed in phosphate buffer (pH=7.4) and the impedance spectrum was measured.

For the formation of the affinity bound complex, 1.5 µl of an appropriate dilution of diluted anti-FLAG, antibody as recomended by the manufacturer (Sigma F 3040, monoclonal mice IgG, Clone M1) and 1 % (v/v) DIEA was added to the immobilized probe-coated array and incubated for 25 minutes. Thereafter, the array surface was washed six times with 500 µl PBS phosphate buffer (pH=7.4) for recording the impedance spectrum.

The impedance measurements were performed as described for Example 1. The Nyquist plot indicated an increased impedance in case of formation of an affinity binding complex on the array.

Example 3: detection of tumor marker CK-20 DNA by impedance measurements and simultaneous electrode processes:

Similar to Example 1 (for the detection of tumor marker CK-20 DNA), (a) a chip with an interdigitated gold electrode array (structured according to Figure 1c), (b) an electrode width of 1µm and (c) an electrode spacing of 0.7 µm was used for probe immobilization. The electrode surface was cleaned with concentrated sulfuric acid for 1 minute, washed with distilled water and then coated with a 1µl solution of 1mM 5'-(SH-hexyl)-CGA TCT GTT TTA TGT AGG TCA-3' (probe DNA) in distilled water by means of self-assembling for 1 hour at room temperature. After washing with distillated water the array immersed into a 20µM

solution of a mixture of $K_3[Fe(CN)_6]$ and $K_2[Fe(CN)_6]$ in phosphate buffer, pH=7.4, and the impedance spectrum was measured.

The coated array was allowed to contact the DNA sample solution in a flow cell, applying the hybridization conditions. Therefore a sample solution containing about 1µmol of the CK-20 DNA, which contained the target sequence 5'-Biotin-TGA CCT AAC CCT ACA TAA AAC AG-3' in 300mM NaCl, 30mM Na-Citrat-buffer, pH=7.0 was used. The surface was treated with 3µl of the solution for 30 minutes at 50°C - 60°C. After washing with 3mM Na-citrat-buffer with 30mM NaCl, pH=7.0 solution, the impedance of the array was measured in a 20µM solution of $K_3[Fe(CN)_6]$ in phosphate buffer (pH=7.4).

The impedance measurements were performed using the frequency response detector and potentiostat/galvanostat, as performed in Example 1. In this example, however, the system was potentiostated at 400 mV vs. open circuit potential. The amplitude of the AC input signal was 10 mV and impedance spectra were recorded in the frequency range of 1 mHz - 1 MHz. The Nyquist plot indicated an increased impedance in case of the formation of the double-stranded DNA on the array.

Example 4: **detection of FLAG antibody by impedance measurements and simultaneous electrode processes:**

For the detection of "FLAG" antibody , the electrode array was prepared as described in Example 2, except that after ester deactivation, the array was washed in distilled water and was immersed into a 20µM solution of $K_3[Fe(CN)_6]$ in phosphate buffer (pH=7.4), followed by the impedance measurement.

Thereafter, 1.5 µl of an appropriate dilution of diluted anti-FLAG was added to the immobilized probe-coated array and

incubated, as described in Example 2. Then, the array surface was washed six times with 500 µl PBS phosphate buffer (pH=7.4) and was immersed in a 20µM solution of $K_4[Fe(CN)_6]$ in phosphate buffer (pH=7.4), and the impedance spectrum was measured.

The impedance measurements were performed using the frequency response detector and potentiostat/galvanostat, as performed in Example. The system was potentiostated at 400 mV vs. open circuit potential. The amplitude of the AC input signal was 10 mV and Impedance spectra were recorded in the frequency range of 1 mHz - 1 MHz. The Nyquist plot indicated an increased impedance in case of formation of an affinity binding complex on the array.